ADDIS ABABA SCIENCE AND TECHNOLOGY UNIVERSITY

College of Biological and Chemical Engineering

Department of Environmental Engineering

PHYTOREMEDIATION OF TANNERY WASTE WATER USING HORIZONTAL SUB SURFACE FLOW CONSTRUCTED WETLAND

A Thesis Submitted to College of Biological and Chemical Engineering of Addis Ababa Science and Technology University in Partial Fulfillment of the Requirements for the Degree of Master of Science in Environmental Engineering

By: Melkamu Bekele

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Declaration

I declare that this thesis is not submitted to any institute other than AASTU and has not been presented by any other person for an award of a degree in this or any other University. Information derived from other works has been duly acknowledged both in the body and the reference lists**.**

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Addis Ababa Science and Technology University Post Graduate Studies

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The thesis Submitted in partial fulfillment of the requirements for the degree of Master of Science in environmental engineering complies with the regulation of the University and meets the standard with respect to originality and quality.

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Acronyms

AASTU	Addis Ababa Science and Technology University
APHA	American Public health Association
AOAC	Association of Official Analytical Chemistry
BAF	Bioaccumulation factor
BOD	Biological Oxygen Demand
COD	Chemical Oxygen Demand
Cd	Cadmium
Cr	Chromium
Cu	Copper
CW	Constructed wetland
EEPA	Ethiopian Environmental Protection Authority
HRT	Hydraulic Retention Time
IS	Indian System
mg/l	milli gram per liter
mg/kg	milli gram per kilo gram
Ni	Nickel
Pb	Lead
SF	Surface flow
SSF	Sub Surface Flow
Zn	Zinc
TF	Translocation factor
TSS	Total Suspended Solid
TN	Total Nitrogen
WW	Waste water

Abstract

*Tannery industries have become a source of environmental pollution due to inadequate treatment of wastewater which contains high organic load and toxic inorganic contaminants. This study was conducted to treat tannery wastewater in subsurface flow constructed wetland using Vetiver grass and Nut Sedge (Nut grass).Together with control unit; seven experimental unit cells were constructed. The wastewater samples were taken at intervals of seven days residence time for analyzing of heavy metals(Cr, Pb, Cd, Zn, Cu, and Ni), TSS, BOD and COD using test method AOAC 974.27, IS 3025,APHA 5210B and APHA 5220B open reflux methods, respectively. The plant samples were digested in microwave digestion for analyzing heavy metals (Cr, Pb, Cd, Zn, Cu, and Ni) in atomic absorption spectro photometry (Graphite method). The waste water analysis showed that the BOD, COD, TSS and Cr of effluents were reduced to 88%, 80.8%, 80.4 % and 97.7 %, respectively in constructed wetland planted with Vetiver grass, whereas*78.5% *BOD,* 73.1% COD, 69.04% TSS and 94.5% of Cr were reduced in control unit. *The statistical analysis ($P< 0.05$) showed that there was a significant difference between the phytoremediating experimental units and control unit for removing selected pollutants. The plant analysis showed that, the chromium metal was largely accumulated in root tissue of both Vetiver grass (55mg/kg) and Nut sedge (33.86 mg/kg). The bioaccumulation capacity (6.7 times) coupled with translocation capacity (0.25 times) of Vetiver grass was higher compared to the Nut grass which has* 3 times of BAF and *0.12 times of TF. As the result, constructed wetland treatment using Vetiver grass is going to be candidate as post treatment method for small scale tannery industry.*

Key words: *Constructed wetland, Phytoremediation, Tannery waste water, Vetiver grass, Nut sedge (Nut grass), Bioaccumulation and Translocation factor.*

Chapter one

Introduction

1.1Background and justification

Water is a basic need of our planet and greatly influences public and environmental health as well as providing the pedestal for most of our economic activities (Mwanyika et al., 2016). The trend of industrial revolution has led to environmental degradation by discharging partially treated and untreated waste water in to aquatic eco system (UNESCO, 2009).

Tanning industries are one of the most manufacturing sectors which are responsible for tremendous pollution of water resources (Siddiqee et al., 2012). Even if, the Ethiopian tannery industries are potential for economic growth and development, they are the great challenge to environment due to inadequate waste treatment facilities. Due to lack of strong regulatory policies, Ethiopian tanneries discharge high load of organic and inorganic pollutants which above tolerance limit to nearby river without adequate treatment facility (Gebre Mariam and Desta, 2002).

In tanning industries, animal hides and skins are transformed in to leather product through different stages which consume much amount of water and large quantity of chemicals including chromium salts (Khan, 2001). According to Ludvick (2000) and Ketema (2009), 30-56 m^3 volume of water is consumed per ton hide processed and 85 % of this water is discharged as wastewater. The physic-chemical method including screening, sedimentation, chemical precipitation ion exchange, electro chemical, adsorption and the biological treatment methods including activated sludge process, sequencing batch reactor, trickling filter are commonly practiced for tannery wastewater treatment (Ketema, 2009).

The physical/chemical treatment method alone is not sufficient enough for removing organic and inorganic pollutants in tannery effluent. Rather, it is expensive and produces secondary pollutants ((USEPA, 1999). High load of chromium in waste water may toxic to microorganism in biological treatment method (Goswami, 2014). The integrated physico-chemical and biological treatment system are essential to comply with the environmental legislation, but this system is expensive, labor and energy intensive, require high maintenance and operational cost (USEPA, 1999 and Ketema, 2009).

Several studies have been attempted to tackle this problem. Constructed wetland is one of the sustainable means of emerging technology which is designed and constructed to treat waste water through biological, physical and chemical means. It comprises vegetation, substrate, microorganism and wastewater (Vymazal, 2011; saeed and sun, 2012).

Phytoremediation is defined as the engineered use of green plants including grass, fobs and woody species to remove contaminants from contaminated soils, ground water and waste water. This technology is highly promising in tropic zone due to prevailing climatic condition which favors plant growth and stimulates microbial activities (Zhang et al., 2010).

In this study, two plant species namely Votive grass (Chrysopogon zizanioides) and Nut sedge (Cyprus rotundus) were selected due to their morphological(fibrous, fine, massive root system) and physiological(resist to different pH, resistant to nutrient load, tolerate to toxicity of heavy metal and adapt different environmental condition) features for treating waste water (Troung, 2003 and Mishra, 2016).

1.2 Statement of the problem

The tannery waste effluent does contain high load of organic matter and inorganic toxic chemicals including chromium which is above the permissible limit standards in Ethiopia (Firdissa et al., 2016). These pose serious negative impacts, not only on surface water bodies and ground water, but also in aquatic ecological system and human welfare. The aquatic ecosystem is going to be deteriorated due to the accumulation of organic and inorganic pollutants. Even if at low concentration, the trace heavy metal including Cr has significant impact on destructing aquatic life as well as human health through ecological food chain .It may expose developmental and neurological disorder, Kidney damage, several type of Cancers for human being and damage the breathing system of aquatic life like fish (Nagajyoti et al.,2010). Batu tannery factory has only primary waste water treatment method. Even if the effluent can't achieve the legal limit standard, the factory is limited to primary treatment. This is because, the cost of conventional post treatment methods are so expensive. Even if once erecting, the treatment plant require high maintenance and operational cost, labor intensive and consume much power during the treatment process (EEPA, 1999). Hence, the Phytoremediation method using Vetiver grass and Nut sedge (Nut grass) in a constructed wetland will be as an alternative solution as a post treatment for Batu tannery factory (which the nearest factory to experimental site)

1.3 Objective

1.3.1 General objective

- To treat Cr and other selected tannery waste water pollutants(BOD, COD, TSS, Pb, Cd, Zn, Cu and Ni) in SSF constructed wetland using Vetiver grass and Nut sedge

1.3.2 Specific objectives

- Characterize the selected tannery waste water pollutants
- Examine the performance of constructed wetland with Vetiver grass and Nut sedge for removal of selected pollutants
- Evaluate and compare the growth performance and chromium accumulation capacity of Votive grass and Nut sedge
- Design and scale up constructed wetland plant for Batu tannery factory based on experimental result

Scope of the study

This study is applied to treat all type of industrial waste water in small scale industry. It is limited to effective wetland substrate and selective green plants to treat liquid waste only excluding solid and gaseous waste that generated from industries.

Significance of the study

The study is significantly used for industries who comply environmental legislation by establishing simple and cost effective treatment system. It is used to attain the environmental sustainability by balancing natural environment and alleviating the problem relating to impacts of industrial waste water.

Chapter two

Literature review

2.1 Characteristics of tannery wastewater

Tannery waste water is characterized with strong alkaline, high salt and chromium content with dark brown color, offensive odor and has high content of organic substance that varies according to the type of process and chemical used (Jahan et al., 2014). It is highly contaminated with Cr, S^{2-}, $SO42^{-}$, suspended solid, BOD_5 and COD content, oil, grease, and chlorides (Suganthi et al., 2013).

There are three main sections for production of leather in tanning industry. These are beam house, tanning and finishing process. High organic matter, total suspended solid and sulfide are generated from beam house operation, whereas the other trace heavy metals and COD are from dyeing and finishing process. In tanning- yard operation, the animal hide and skin are converted to strong and stable leather product through reaction of chromium with animal hide and skin. At least 300kg chemicals are used per ton of hide in tanning process and hence, huge waste water has been generated with high load of COD and Cr content (Midha et al., 2008).

2.2 Environmental Impacts of tannery waste water

Environmental pollution is an inevitable consequence of people's pursuit of better life and economic development through industrialization. It is a contamination of environment with the presence of pollutants which above are the permissible limit in the environment. The industries have mainly contributed for generation of these pollutants that cause objectionable effects, impairing the welfare of environment, reducing the quality of life and also cause to death (Nagajyoti et al., 2010).

Tanning industries are among the polluting industries which discharge high level of pollutants and these poses serious environmental impacts on aquatic, terrestrial and atmospheric system (Songet et al., 2009). Due to lack of strong regulatory policies, Ethiopian tanneries discharge high load of organic and inorganic pollutants which above tolerance limit to nearby river without adequate treatment facility (Gebre Mariam and Desta, 2002).

2.2.1 Impacts of organic pollutants

When High load BOD_5discharged to river, the much populated bacteria will grow up and require proportional free DO to degrade organic matter. It leads to stress aquatic organism including fish due to lack of free available DO. Likewise BOD_5, TSS also has significant impact on aquatic life. The concentrated TSS may cause to scatters sun light, impaired photosynthetic activities of algae and stress aquatic animals.

2.2.2 Impacts of Heavy metals

The term Heavy metal is any metallic element including both metal and metalloid which are toxic even at low concentration (Nagajyoti et al., 2010). Some heavy metals are bio-accumulative and persistent which neither break down nor easily metabolize in the environment. Such heavy metals accumulate in ecological food chain through uptake of primary producer and through consumer. High exposure of this metal leads to lung cancer, damage kidney, heart and central nervous system of human being (Reza and Singh, 2009).

The concentrated level of heavy metals including Cr, Zn, Cd, and Pb are highly toxic to plants. It induces physiological and morphological disorder of plant and may cause to retardation of plant growth, chlorosis, necrosis and eventually senescence, since concentrated level of metals close stomata, hinder the movement of water and nutrient and inhibit metabolism (Nagajyoti et al., 2010).

2.2.2.1 Impacts of Chromium and its chemistry

Chromium is one of the major pollutants from tannery industry and it is the transitional metal which exists with different oxidation state (from Cr^{+} _ Cr^{+6}). The trivalent chromium (Cr^{+3}) and hexavalent chromium (Cr^{+6}) are the most abundant form. Except Cr^{+3}, the remains are unstable ((Terfie and Asfaw, 2015). Cr is removed from the solution as Cr (OH) 3.aq. (Trihedral chromium) which is slightly soluble. It is amphoteric oxide (slightly acidic) which is changed to tetra hydro chromium complex (Cr (OH) $_4$ at high pH. If Fe is present in the solution, Cr is removed as (Cr_xFe_{1-x}) (OH) $_3$, where x is mole fraction of chromium. The effect of chromium depends on concentration of chromium, organ at risk and the exposure. It may cause to acute and chronic effects based on its concentration and duration. It may cause to skin, lung cancer, damage kidney and chronic toxic effects on aquatic life. Cr^{+6} has carcinogenic, and gene toxic effect to human being (Achmad et al., 2017). EEPA declare 2mg/l Cr concentration for discharging river water (EEPA, 2003).

2.4 Tannery waste water treatment methods

2.4.1 Conventional treatment methods

Physico-chemical and biological treatment methods are used to remove organic and inorganic pollutants in tannery industries. Physico-chemical methods including advanced oxidation, coagulation and flocculation, chemical precipitation, adsorption and ion exchange method, membrane filtration and reverse osmosis are commonly practiced for removal of tannery waste water pollutants before discharging to nearby river. Even if the conventional methods are commonly practiced, they are so expensive in capital and maintenance cost as well as produces secondary pollutants (USEPA, 1999).

Trickling filter, activated sludge, sequencing batch reactor and stabilization ponds are conventional biological treatment method which used for degrade and remove organic and inorganic pollutants. The trickling filter which is formed by a layer of filter medium held within a containing tank or vessel. The filter is designed to permit good drainage and ventilation. The microbial growth occurs on the subsurface of stone or plastic media and the wastewater passes over the media along with air to provide oxygen, whereas the activated sludge process is a continuous or semi continuous process which containing activated microorganism for stabilizing organic matter (Terfie and Asfaw, 2015).

2.4.2 Waste water treatment methods in Batu tannery factory

Batu tannery factory has three main sections (beam house, tanning yard and finishing operation) for producing leather product. The waste water is generated from this manufacturing section. The factory has primary treatment methods for treating generated waste from three different lines.

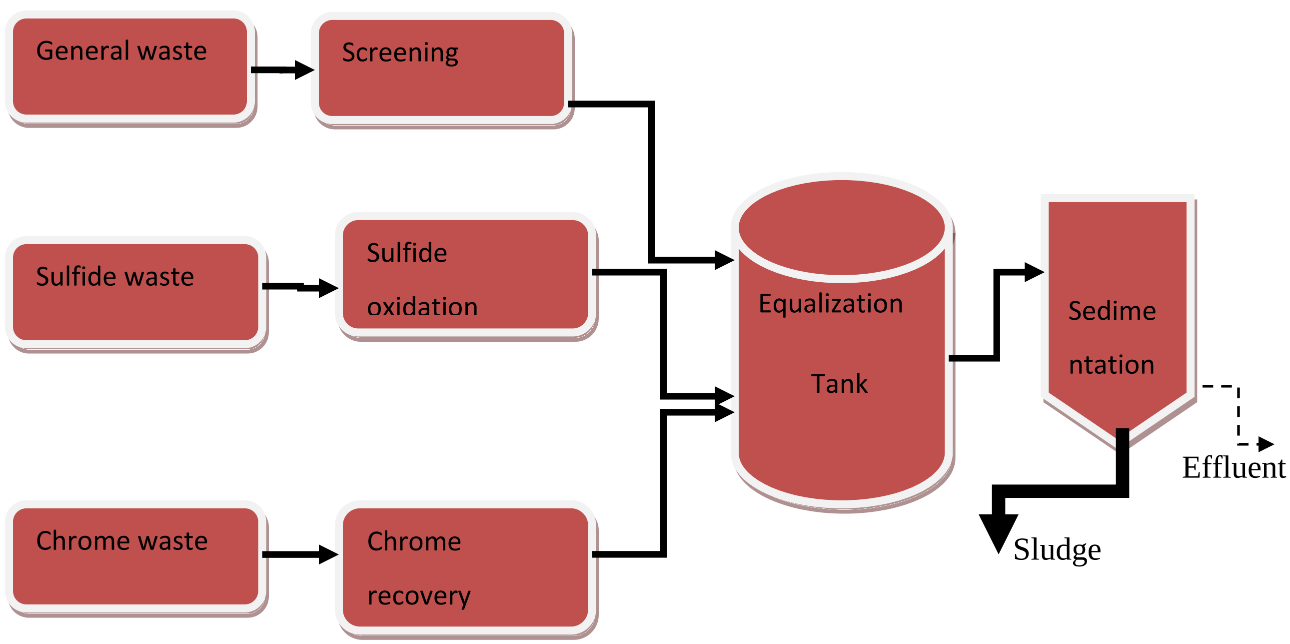

Figure 2.1: Wastewater treatment line of Batu tannery factory.

The waste water that generated from the three sections is homogenized in equalization tank. After precipitation, the sludge waste is disposed down from sedimentation tank, whereas effluent waste is discharged to environment (Small Akaki River) through discharge pipe.

2.5 Constructed wetland

Wetland: Wetland is a transitional area between land and water. The term "wetland" encompasses a broad range of wet environment, including marshes, bogs, swamps, wet meadows and floodplains (USEPA, 1993).

Wetland can be classified as natural wetland and constructed wetland. Constructed wetland is artificial, engineered wetland which is designed and constructed to mimic natural wetland system for treating wastewater. This system mainly encompasses water, plants, substrate, bacteria and nutrients. In this constructed wetland, the pollutants are removed from wastewater in physical, chemical and biological means (Wu et al., 2015). It is a green treatment technology which enhancing natural wetland for treating various types of wastewater including domestic, industrial, drainage, storm water and polluted river (Saeed and Sun, 2012).

Besides water quality improvement and energy saving, constructed wetland has other environmental protection features such as promoting biodiversity, providing habitat for wetland organisms and wildlife (Raskin et al., 1997). This method generally cost effective, simple and environmentally non-disruptive, ecologically sound with low maintenance cost. Natural wetland has been as waste water discharge site, since the beginning of sewage collection in 1950 in Germany .The first free water surface constructed wetland was implemented in Netherlands in 1967, whereas the first sub surface flow constructed wetland was discovered in 1974 for municipal wastewater treatment in Germany (Wu et al., 2015). Traditionally, CWs have been used as sewage treatment since, 1980 (Kadlec and Knight, 1996).

2.5.1 Types of constructed wetland

Constructed wetland can be classified as:

1. Based on Vegetative plants: according to Brix, 1993, based on life form of dominant macrophyte constructed wetland may be grouped in to:

a) Rooted emergent plant based system

b) submerged plant based system

c) Free floating plant based system

2. Based on hydrology: according to USEPA, 1993. Constructed wetland mainly grouped into:

1) surface flow constructed wetland

2) Subsurface flow constructed wetland

3) Hybrid flow constructed wetland.

Surface flow constructed wetland: Surface flow constructed wetland consists of a shallow basin that fills with soil or peat for support plant growth. In the surface flow constructed wetland, the water is exposed to the environment, since the water flow over saturated surface.

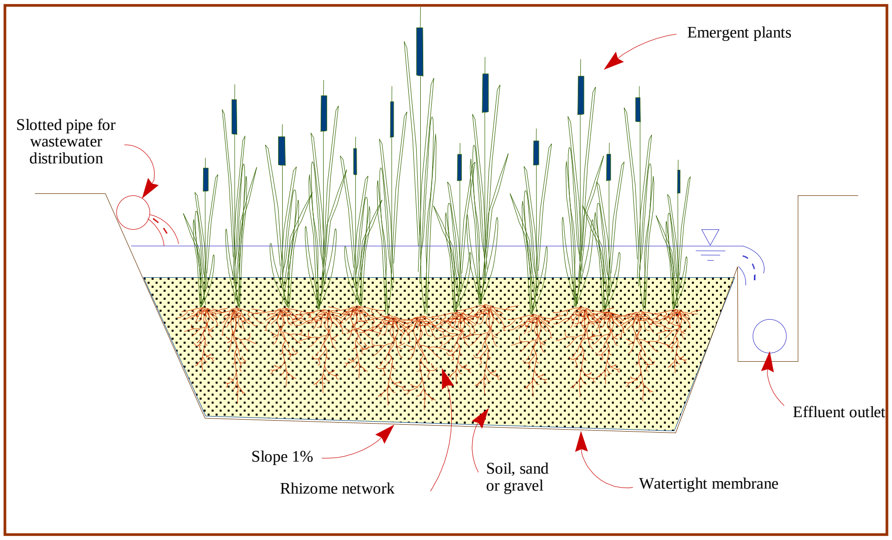

Figure 2.2 surface flow system (Diederik Rousseau, 2011)

Subsurface flow constructed wetland: It is a properly sealed basin which fills with porous medium. The water level designed just below the top of the substrate and flow through the porous medium. The water is not exposed to the environment. It demonstrate high rate of contaminant removal per unit of land than surface flow. SSF constructed wetland is smaller while achieving the same level of contaminant level. This reduces human and ecological exposure. Based on water flow, this constructed wetland is grouped in to vertical flow and horizontal flow constructed wetland.

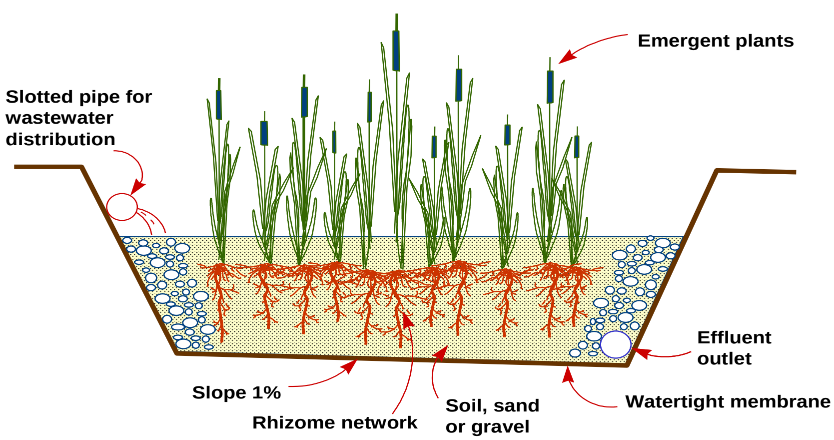

Figure 2.3 horizontal sub surface flow constructed wetland (Diederik Rousseau, 2011)

Hybrid flow constructed wetland system

Various types of constructed wetlands may be combined in order to achieve higher treatment effect especially for nitrogen. It mostly comprises vertical flow and horizontal flow arranged in staged manner (Vymazal, 2005).

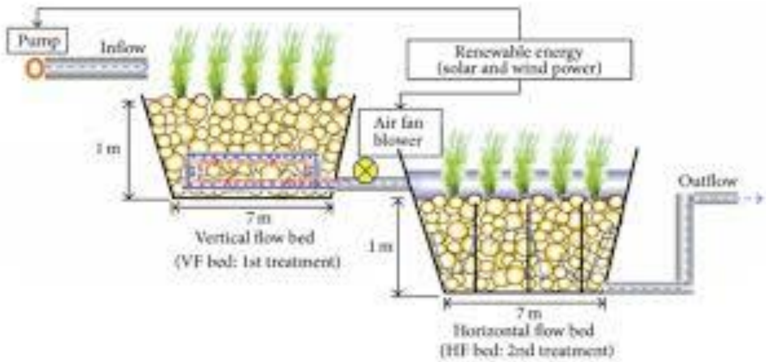

Figure 2.4 hybrid constructed wetland system

As shown in figure 2.4, the waste water sprayed out to vertical flow constructed wetland using pressure pump. The treated wastewater from vertical flow constructed wetland is fed to horizontal flow constructed wetland for further treatment. The effluent waste of vertical flow constructed wetland will be the influent waste of horizontal flow CW system.

Advantages of constructed wetland over conventional treatment

1. Low capital, operational and maintenance cost
2. Low energy consumption
3. Balancing natural environment
4. Enhance aesthetics of open space
5. Provide habitat for plants and wild life
6. Applicable any type of waste water

Advantages of SSF CW over SF CW (Halverson, 2004).

A. High rate of contaminant removal per unit of land than SF CW

B. Require less land to achieve a particular level of treatment than SF

C. Minimal ecological risk due to the absence of an exposure pathway.

D. No Odor and insect problem, since water level is below medium surface

2.5.2 Components of constructed wetland and pollutant removal mechanism

Constructed wetland is mainly composed of selective green plants, effective substrate, polluted water and microbes for improving water quality.

Substrate: The constructed wetland substrate mainly used to support plant growth and enhance microbial interaction with in the system. The substrate which used for constructed wetland may differ with different waste water, type of constructed wetland and vegetation. The substrates may be soil, sand, gravel, crushed rock and industrial by products. The selection of substrate is based on hydraulic permeability and pollutant absorbing capacity. Poor hydraulic conductivity would result in clogging the system and decrease the efficiency of CWs. The low absorbing capacity of substrate cause to slow removal system of CW (Wang et al., 2010). The pollutant removal system also affected by size of substrate. Many chemical and biological transformations take place within substrate which affects the movement of water and provide storage for many contaminants. The substrate provide for removing pollutants through cat ion exchange, adsorption, precipitation and complexation mechanism (Lai and Lamb, 2009).

Microorganisms: A fundamental characteristic of wetland and its functions is largely regulated by microorganisms and their metabolism (Wetzel, 1993). Microorganisms include bacteria, yeasts, fungi, protozoa, and algae. Microorganism transforms complex organic into simple substances through aerobic or anaerobic condition. Some microbial transformations are aerobic (they require free oxygen) while others are anaerobic (they take place in the absence of free oxygen). Many bacterial species are facultative which are capable of functioning under both aerobic and anaerobic conditions in response to changing environmental conditions (Hilton, 1993). The microbial community of a constructed wetland can be affected by toxic substances, such heavy metal. So, great attention must be taken to prevent such chemicals.

Suspended and attached microbes are responsible to degrade soluble organic compounds, either aerobically or an aerobically. The oxygen require for aerobic degradation is supplied directly from the atmosphere by diffusion or oxygen leakage from plant roots into the rhizosphere. Aerobic degradation of soluble organic matter is governed by aerobic heterotrophic bacteria according to the following reaction.

$$(CH_2O) + O_2 \longrightarrow CO_2 + H_2O$$

The autotrophic group bacteria which degrade organic compounds contains nitrogen under aerobic condition is called nitrifying bacteria and the process is called nitrification.

Microbial mediated process: The metal oxidizing bacteria in aerobic condition and the sulfate reducing bacteria in anaerobic condition will cause precipitation of metal oxides and sulfides respectively (Vymazal, 1995). In Plant root interface, there are very steep redox gradients which resulting the precipitation of metal oxy hydroxides. These precipitate may provide an effective barrier restricting metal uptake through co-precipitation of other heavy metals (ye et al., 1994).

Wetland plants: Wetland plants are green plants that grow in wetland with or without support of substrate. The wetland plants have great contribution for remediating waste water and enhancing the water quality through different ways. The way of remediating contaminants using wetland plants from contaminated site is called Phytoremediation.

2.5.3 Phytoremediation

Phytoremediation is engineered use of selective green plants to remediate organic and inorganic pollutants from contaminated sites including waste water and contaminated soil (wu et al 2015). The word Phytoremediation comprised from the prefix Greek word phyto (meaning plant) and the suffix Latin word remedium meaning clean up evil (Cunningham et al., 1996). It can be used along with conventional treatment technology (as alternatives) or in place of conventional treatment (as complementary technology) (Etim, 2012). It is a mechanism to remove contaminants from nature using selective plant species. In broad sense, the term phytoremediation is a process to reduce volume, toxicity and mobility of pollutants from environment (USEPA, 2000). It is non-destructive, environmentally sound, cost effective insitu technology to remove contaminants from polluted environment (Etim, 2012)

2.5.3.1 Mechanism of Phytoremediation

The mechanism and efficiency of phytoremediation depend on the type of pollutants, bioavailability and the property of contaminant site (Cunningham, 1996). The Phytoremediation mechanisms are:

1. Phytoextraction
2. Phytostabilization
3. Phytodegradation
4. Phytovolatilization
5. Rhizodegradation
6. Rhizofiltration

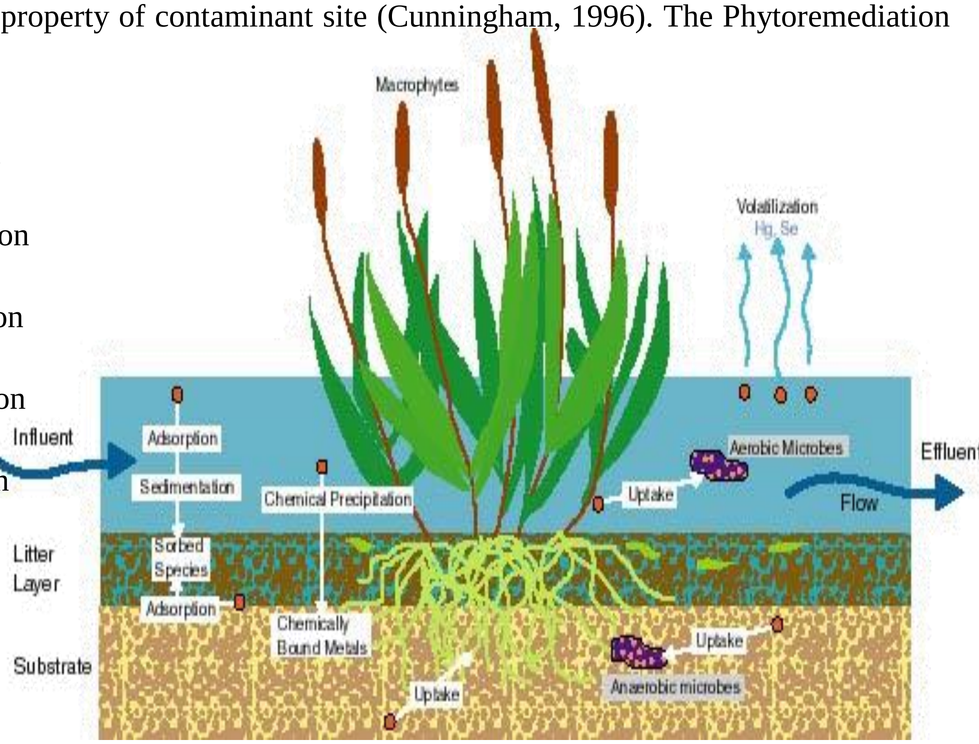

Figure 2.5 Phytoremediation mechanisms (Halverson, 2004)

Plant roots are the versatile parts of plant system that provides enormous surface area to absorb and accumulate water nutrients and other pollutants. The Phytoremediation technology is potential in tropic zone due to prevailing climatic condition that favors plant growth and stimulates bacterial activity (Zhang et al., 2010).

Phytoextraction (phytoaccumulation): It is the absorption and translocation of metal contaminants by plant root in to above ground portion of the plant. There are several factors limiting to phytoextraction mechanism (EPA, 2000).

a) Bioavailability of heavy metals with rhizosphere

b) Rate of heavy metal uptake by roots

c) Cellular tolerance of heavy metal toxicity

d) Proportion of heavy metal with root

To be feasible phytoextraction method, it should be:

- Extract heavy metal
- Translocate heavy metals to above ground
- Produce large quantity of mass

Adsorption and cat ion exchange: It is the binding of heavy metal on to plant surface or matrix surface. In cationic exchange reaction, the positively charged metal ions in solution bind to negative charged ion on the surface of adsorption material. The size of cationic exchange force and surface area of vegetation are the major factor in the process (USEPA, 2000).

Phytodegradation: It refers to phytotransformation which is the degradation of complex contaminated molecules into simple molecules with in plant tissue after up taking from its root (USEPA, 2000).

Rhizodegradation: It refers to phytostimulation which is a breakdown of organic contaminants with in plant root zone by microbes. Microorganism may be prevalent in the rhizosphere, because the plant exudes sugars, amino acids, enzymes and other compounds that stimulate bacterial growth (USEPA, 2000). Additionally, it provides surface area for microbial growth and pathway oxygen transfer.

2.5.4Classification of wetland plants

Generally, there are three groups of wetland plants (Phytoremedial plants) that are commonly used for waste water treatment process in proper design constructed wetland (Wu et al., 2015).

1) Free floating plants
2) Submerged plants
3) Emergent plants

Free floating plants: Free floating plants including Water Hychin, Duckweed, Azzola which are grown freely on the surface of water in constructed wetland (Vymazal, 2013b).

These plants are mostly used for removing contaminants from waste water by using their root system through different mechanism including adsorption and absorption.

Submerged plants: It includes Egeriadensa (dense water weed), Elodeanuttallii (Water weed) are grown with in water in constructed wetlands (Vymazal, 1995). Most of their structure found below the water. Their photosynthetic tissues are entirely submerged with in water and hence the turbidity of waste water must be low, because high turbidity blocks the transmission of light to plants (USEPA, 1988). The plants depletes the dissolved organic carbon in water and increase the dissolved oxygen ,this lead to increment of pH value and volatilization of ammonia & chemical precipitation. High oxygen concentration also creates favorable condition for mineralization. The nutrients removed by plants are largely retain with in root tissue of plants and by attached Micro flora.

Emergent plants: emergent plants are the most widely used vegetation plants in subsurface and surface flow constructed wetlands, including Phragmites, Typha latifolia, Vetiver grass, Nut grass which are grown with support of substrate. They have large portion of their shoots, leaves or flowering structure out of the water (Vymazal, 2011). The pollutant removal mechanisms are carried out through adsorption, absorption, filtration, rhizodegradation, plant up taking.

Selection of wetland plants

The major selection criteria of wetland plants are

1. Physiological and morphological nature of the plants
2. Resistant to high nutrient load
3. Resist to toxicity
4. Resistant to extreme pH(basity and acidity)
5. Resistant to extreme environmental condition (extreme temperature and cold)
6. Sub-tropical plants (the environment favorable for concerned plants)
7. Adaptive plants in constructed wetlands

2.6 Experimental plants

2.6.1Vetiver grass

Vetiver grass (Chrysopogon zizanioides) is a perennial grass that belongs to the gramineae family. The Vetiver grass is a unique tropical plant that has been proven and used in some 100 countries for soil and water conservation, land rehabilitation, pollution control, water quality improvement and many other environmental applications. It has deep dense, fast-growing, spongy long root system which capable of reaching 3.6 m. This provides for bacterial and fungal growth and multiplication which are required to absorb and to break down contaminants within the processes (Truong, 2003).

It has the ability to resist acidity, alkalinity, salinity and heavy metals. Vetiver can withstand drought and is not affected by flood. Although it is a tropical grass, it can also tolerate extreme temperatures from -15 ℃ to 60 ℃ (Troung, 2008).

2.6.2 Nut sedge (Nut grass)

Nut Sedge (Cyperus rotundus) is commonly known as purple Nut sedge, Nut grass, Red nut sedge, Coco grass, Water grass which is belong to Cypraceae or Sedge family, native to Africa, Southern and Central Europe(Mishra,2016). It is perennial erective stem flowering plant which grows in polluted wet environment. It has dark brown tuber, white fleshy root system and then form a bulb- like structure from which new root and shoot grow (Mishra and Chauhan, 2013). Cyperus rotundus can tolerate salt stress and has the ability to accumulate Chromium, Manganese, iron, copper and Zink in some wetlands which emphasizes the importance of phytoremediation as a cost effective tool for environmental cleaning (Chatterjee et al., 2011). This species also accumulate iron, chromium, lead, copper, and cadmium in root system (Mishra, 2016). It branched and multiplies its stem and produce large biomass during growth period. It consumes much water for growth and reproduction and widely available throughout the country and mostly used as house decoration system on Holy Day.

Chapter three

Materials and methods

3.1 Study area

The constructed wetland experiment using two phytoremedial plant species for tannery waste water treatment was conducted at Addis Ababa Science and Technology University which is located at 08° 53'099" latitude and 038°48′392" longitude with Altitude of 2137m above sea level. It is 27 km far from Addis Ababa to the South. The tannery wastewater was sourced and transported from Batu tannery factory which is 10 km from AASTU. The Vetiver grass and Nut sedge were taken from AASTU phytoremediation project nursery site which is near to experimental site. The study was carried out under greenhouse condition for total seven months periods.

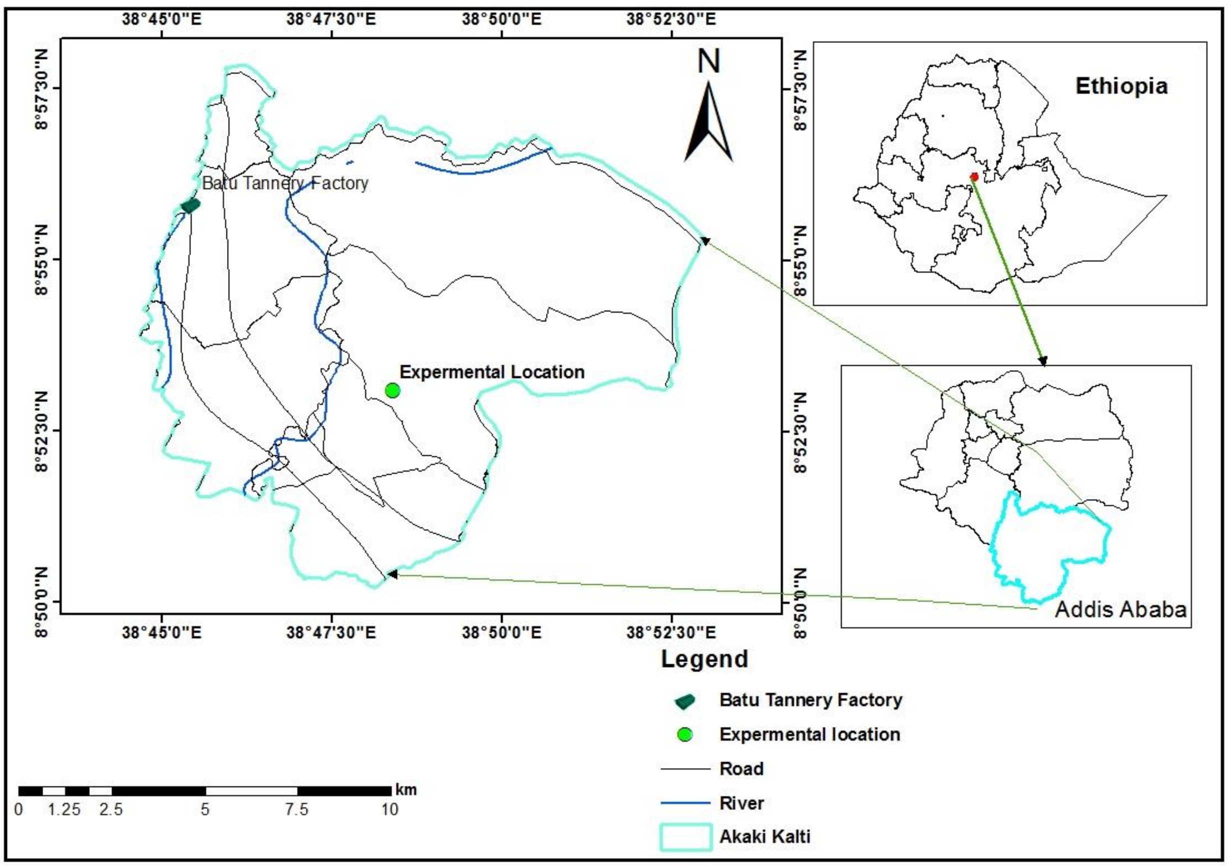

Figure 3.1 location of study area

3.2 Experimental design and set up

The study was carried out through seven pilot constructed wetland cells. Including control unit, each unit cell has 2.85 m length, 0.57 m width, 0.65 m depth and volume of 1.05 m^3. The dimension of length and width were made with aspect ratio 5:1 and its size is depends on the flow rate of wastewater which fed into CW. Each wetland cell was filled 45cm with medium size gravel (15-35 mm) above the cemented base and then 5cm with fine sized gravel (0-2mm) above medium size. The coarse size gravels were used at inlet and outlet zone to avoid any clogging which might be occurred within the wetland cell and through discharge pipe.

The Vetiver and Nut sedge were planted in to their own constructed wetland cells with horizontal subsurface flow system. From seven pilot constructed wetlands, the three triplicate wetland cells were used for evaluating the growth performance of Nut sedge and its efficiency for removing pollutants, whereas the remain three triplicate were used for evaluating Vetiver grass. The control unit cell was filled only with gravel medium without any phytoremedial plant. The wastewater was transported from Batu and fed to each wetland cells with in every seven days interval.

The average operating volume of each unit cell can be calculated using Darcy's formula.

$Vop = L \times W \times d$ ……………………………………………………………….equation 3.1

Where Vop = average operating volume (m^3)

L=length of CW (in m)

W=width of CW (in m)

d=depth of wastewater (in m), It is recommended depth in the case (miller, 2007)

$Vop = L \times W \times d = 2.85 \times 0.57 \times 0.45$

$= 0.731025\ m^3 = 731L$

The total volume of waste water required for each wetland cells can be calculated as

$Vop = L \times W \times d \times n = Vop \times n$ Where n is porosity (35%= n = V_{void} / V_{total}) (USEPA, 1993 and EPA, 1988).

$= 731 \times 0.35/batch$ = 255.8 L/batch/unit cell

Totally, 1791 L/batch of wastewater was required for all seven experimental cells.

Thus, total 1791 L of wastewater were collected in a plastic tank from Batu tannery wastewater discharge site, at every week after noon and transported to AASTU experimental site for four months. The wastewater was equalized in a plastic tank and distributed to each experimental unit cell through distribution tank.

Experimental set up

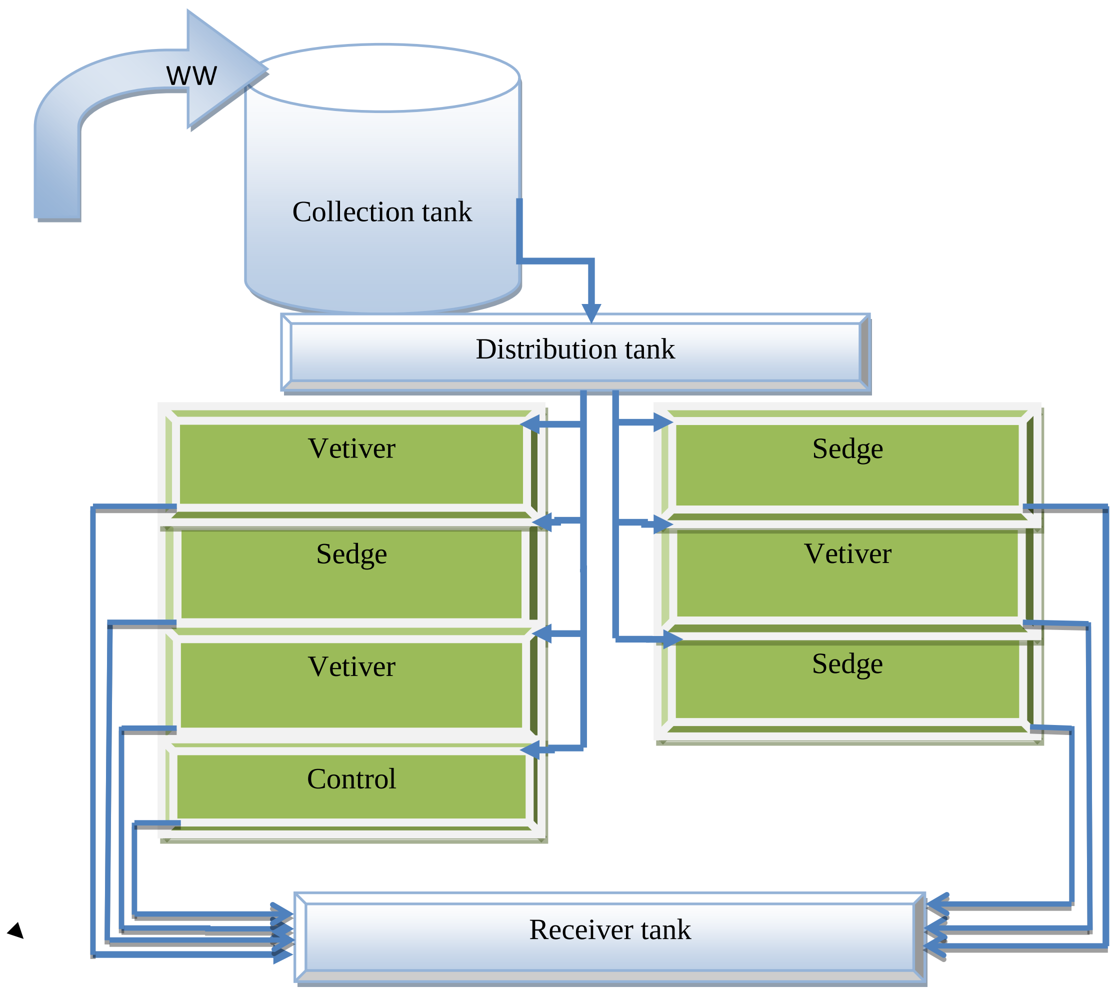

Fig 3.2 Experimental set up, treatment arrangement and randomization techniques

3.3 Transplanting and acclimatization

The Vetiver grass (Chrysopogon zizanioides) and Nut sedge (Cyprus rotundus) were transplanted from AASTU Phytoremediation project Nursery site. Before planting, the Vetiver grass and Nut sedge were trimmed for 10 cm stem height and 5 cm root length after dividing into two tillers per clumps (Dhanya and Jaya, 2013). After trimmed, both Vetiver grass and Nut sedge were planted on its own CW cells with 15 cm×20 cm plant spacing through vertical versus horizontal line (Geovanap et al., 2016)

Figure 3.3 constructed wetland before, during and after planting

The experimental plants were acclimatized in constructed wetland by irrigating tap water for three months. To avoid any plant shocking, the dilute waste water was introduced to CW for three weeks with (70 % Tap water) and for one month with dilution factor two (50% tap water and 50% waste water). The phytoremedial plants were allowed to become well established before the undiluted waste water introduced into the process, to survive stress (USEPA, 1993). The experiment was conducted for two months by feeding undiluted wastewater to each unit cell.

Table 3.1 the ratio of tap water and waste water for experiment

Days	Tap water (%)	Waste water (%)
30/01/2017 - 07/05/2017	100	-
08/05/2017 -28/05/2017	70	30
29/06/2017 -01/07/2017	50	50
02/07/2017-06/09/2017	-	100

The morphological and physiological characteristics of plants were recorded during experiment.

3.4 Experimental analysis

3.4.1 Waste water sample collection and analysis

One liter of wastewater samples was collected in plastic bottles which were washed with tap water and rinsed with distill water before collecting samples to avoid any inherited contamination. The influent samples were taken from equalization tank just before feeding to wetland cells through distribution tank and effluents samples were taken just discharging from each unit cell at interval of seven days residence time. Total 8 influents and 56 effluents were collected throughout the experiment. Then these samples were analyzed for heavy metals *(*Cr, Pb, Cd, Zn, Cu, and Ni), TSS, BOD_5 and COD using test method AOAC 974.27, IS 3025,APHA 5210 B, APHA 5220 B open reflux methods, respectively. The COD and BOD_5 parameters were conducted at Jije Labo Glass Analytical Laboratory service PLC, whereas the TSS and heavy metals were carried out at Bless Agri food accredited laboratory service P.L.C.

The removal efficiency of treatment system for selected parameters can be calculated using the formula (Terfie and Asfaw, 2015).

Removal efficiency $= \frac{\mathrm{Ci-Cf}}{\mathrm{Ci}} \times 100$……………………………………….…………equation 3.2

Where, Ci = initial concentration

Cf= final concentration

3.4.2 Plant sample preparation and analysis

The Vetiver grass and Nut sedge samples were collected before starting up the experiment for pre-test analysis during transplanting time. Three Plant samples were collected from each experimental unit cell at inlet side, outlet side and middle position after seven months experiment for post-test analysis. After leveling, the stem height, root length and total length of living plants (biomass) were measured properly and the average measured values were taken for both Vetiver grass and Nut sedge. After measuring, each samples was washed with tap water and distill water and then dried on oven drier until maintain constant weight. The dry weight of each sample was measured and taken average value. After measuring biomass dry weight, the stem and root weight also measured after separating carefully into stem and root parts. The three root samples itself and three stem samples itself were mixed separately and ground. The stem, root and biomass (whole part of plant) samples were digested in micro wave digestion separately. The heavy metals *(*Cr, Pb, Cd, Zn, Cu, and Ni*)* were analyzed with Atomic absorption Spectro photometry (Graphite method) at Bless Agri Food Accredited Laboratory Service PLC.

Bioaccumulation factor index (BAF)

It is an index of the ability of the plant to accumulate particular metals with respect to its concentration to surrounding medium (waste water). It can be calculated using the following formula (Mellem et al., 2009 and Kastratovic et al., 2014).

$$\text{BAF} = \frac{\textbf{Metal concentration in plant Tissue}}{\textbf{Intial concentration of metal in srounding medium}(\textbf{waste water})} \text{ ....equation 3.3}$$

Translocation factor (TF)

It is an index of the ability of plant to translocate metals from root to Arial parts of plant (Mellem et al., 2009 and Kastratovic et al., 2014).

$$\text{TF}=\frac{\text{Metal concentration in stem}}{\text{Metal concentration in root}}\text{..equation 3.4}$$

3.4.3 Statistical data analysis

The statistical analysis was performed using Design expert program (version 6.0.8). The data was analyzed through one way analysis of variance (ANOVA) for selected factorial model at 95% confident level to compute mean of removal efficiency and compare the performance of CW treatment unit cells for removing selected pollutants .

The significance difference between treatment unit cells determined by evaluating the value of prob> t value. If the value of “prob>t” less than 0.05 then the difference in the treatment efficiency is significance, whereas the “prob>t” greater than 0.1 indicates the difference in treatment efficiency is not significant.

Chapter four

Results and discussion

In this study, the performance of Vetiver grass and Nut sedge plants were evaluated on the base of selected pollutants that being reduced in constructed wetland.

4.1 Waste water characterization

Before introducing in to constructed wetland treatment system, the waste water was being characterized. The waste effluent which discharged from Batu tannery was highly comprised with Cr other than heavy metals, because, chromium metal is highly consumed as raw material in tanning operation to convert putrefy animal hide and skin to stable leather product. The value of remain heavy metals including cadmium (Cd), nickel (Ni), lead (Pb), copper (Cu) and Zink (Zn) were insignificant (<0.1). The concentration of Cr and other selected organic pollutants were above the permissible limit standard in Ethiopia and its concentration varied due to the different chemicals used, amount of water used and type of process. The characterization of wastewater which discharged from Batu tannery factory was shown in table below.

Table 4.1 characterization of waste water used for CW (average influents for two month periods)

No	Parameters (mg/l)	Mean $\pm$SD (mg/l)	Max value(mg/l)	Min value (mg/l)	Max permissible limit (mg/l)
1	BOD	503$\pm$171	630.43	227	200
2	COD	1046.4$\pm$228	1197.76	1107	500
3	TSS	670.4$\pm$372	1172	256	50
4	Cr	4.6$\pm$2.2	7.8	2.31	2
5	Zn,Ni,Cu,Cd&Pb	<0.1	<0.1	<0.1	-
6	PH	7.9	7.6	8.2	6-9

The waste water characterization showed that, average 503 mg/l concentration of BOD and 1046 mg/l COD were discharged to the environment from Batu tannery. This high load of BOD which is beyond the tolerance limit may cause to stress aquatic life due to insufficient amount of free available dissolved oxygen (DO).

Relatively, high amount of COD was discharged compared to BOD. The COD**:** BOD ratio is an important indicator to distinguish the pollutants whether biologically degradable or not. According to (Rehm, 1999), if COD: BOD ratio is greater than two (>2), the waste water is not easily degradable. Hence, the waste water discharged from Batu tannery is not easily biologically degradable, Since COD: BOD ratio is greater than two (>2).

The organic loading rate of wastewater (OLR) was 101 lb/d.arce and the Hydraulic loading rate of wastewater was 0.07 feet/d.

The concentration of chromium (4.6 mg/l) was above the permissible limit standards. It may cause toxic effect on aquatic life and also on human being through ecological food chain. It has carcinogenic and mutagenic property which may cause to cancer and disorder gene function. Since, it is bio accumulative and non-degradable, it may also cause to kidney damage and nerve system (Reza and Singh, 2009).

The higher concentration of TSS also cause to negative impacts on aquatic life. The aquatic life is going to be stressed due to concentrated TSS which scatters sun light and blocks the photosynthetic activity. As the result, the waste water which discharged from source needs further treatment before discharge to the environment. The constructed wetland treatment is the best alternative technology in the case.

4.2 The performance of constructed wetland with Vetiver grass and Nut sedge for removing organic and inorganic pollutants

4.2.1 Removal of organic pollutants in constructed wetland (BOD, COD and TSS)

In this study, the concentration of BOD_5 and COD showed remarkable reduction in constructed wetland. The average value of effluent BOD_5 was 60.36 mg/l, 72.528 mg/l, 108.1 mg/l and COD value was 201.4185 mg/l, 267.402 mg/l, 281.03 mg/l with in Vetiver unit, Sedge unit and control unit respectively.

The removal efficiency of constructed wetland with Vetiver and Nut sedge for removing BOD_5 and COD were shown below in figure.

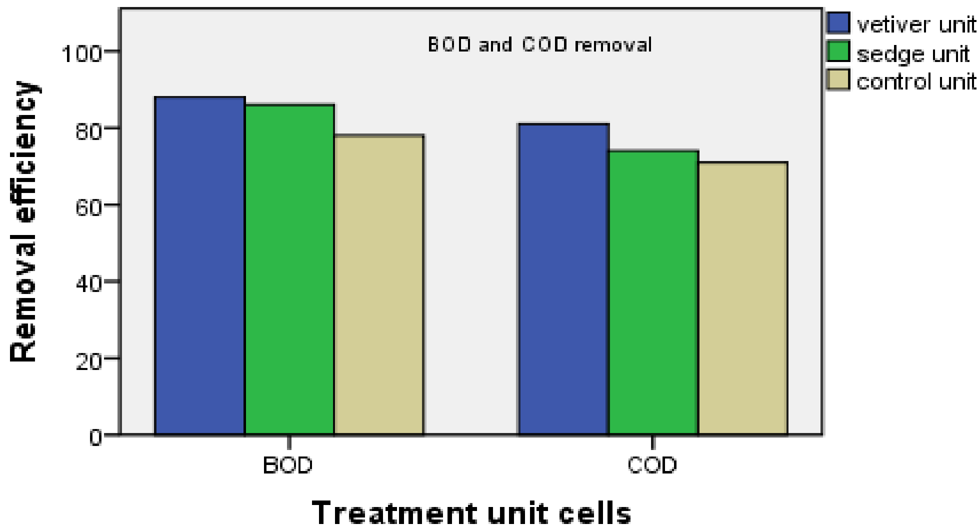

Figure 4.1 BOD_5 and COD removal in constructed wetland

As shown fig 4.1 the maximum BOD_5removal was observed in treatment unit cell planted with Vetiver grass (88 %) and followed by Nut sedge (85.6 %). The minimum removal efficiency was observed in control unit cell (78.5 %). The Statistical analysis ($P<0.05$) showed that, the BOD_5 removal in CW unit cell planted with Vetiver grass was significantly higher than Nut sedge and control unit. There was no any statistical difference between Nut sedge and control unit for BOD_5 removal.

The Vetiver grass had better removal efficiency for COD removal (80.8 %) than Nut grass which was 74.5 % removal efficiency. The lower removal efficiency was recorded in control unit cell (71.4%). The statistical analysis showed that, the Vetiver grass in CW was higher COD removal than other unit cells. There was no statically difference between CW unit cell planted Nut sedge and the control unit.

Likewise BOD_5 and COD, the highest removal of TSS was observed in treatment unit cell planted with Vetiver grass (80.4 %) and followed by Nut grass (79%). The lowest removal of TSS was recorded on control unit (69 %).

The analysis showed that, there was no any significance difference within treatment unit cells.

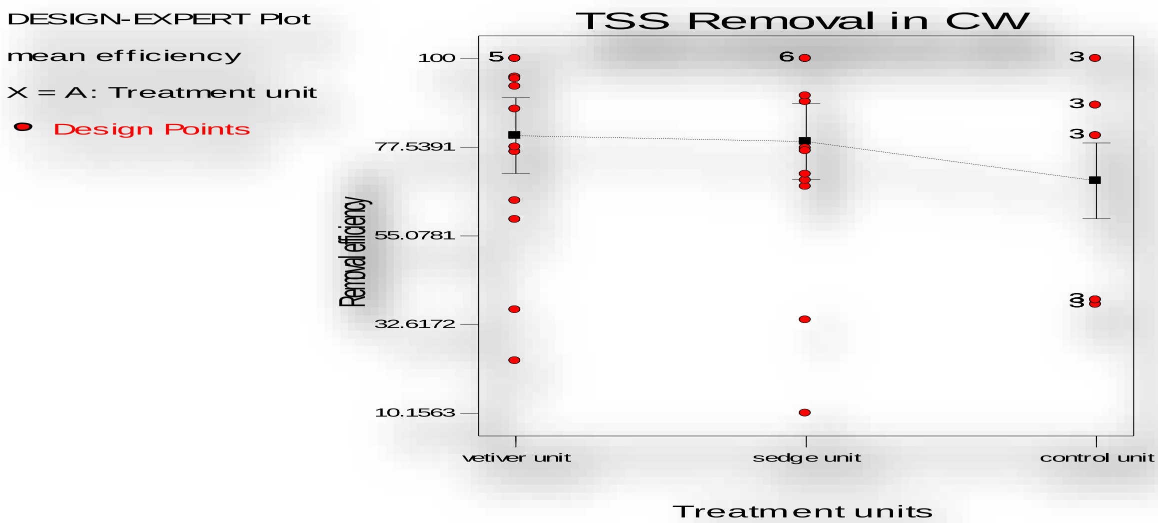

Figure 4.2: TSS removal in constructed wetland

From fig 4.1 and fig 4.2: It can be seen that, the capacity of vegetative unit cells for removing organic pollutants were potentially higher than control unit cell. This was due to vegetation which provided for remediating contaminants through different mechanisms including Rhizodegradation, phytodegradation and phytovolatilization (Etim, 2012). The organic contaminants are degraded with in plant root zone through Rhizodegradation mechanism. Microorganism may be so prevalent in the rhizospher, because the plant exudes sugar, amino acids, enzymes and other compounds that can stimulate bacterial growth. The root also provides additional surface area for microbes to grown and pathway for oxygen transfer from the environment (Zhang et al., 2010). Mostly the organic pollutants including polycyclic hydrocarbons(PAHs), chlorinated solvents, polychlorinated biphenyls (PCBs), benzene, toluene and ethyl benzenes are degraded in rhizodegradation mechanism (EPA,2000). The plants remove volatilized organic compounds including trichloroethen through phytovolitization process. It involves taking up contaminants from contaminated site, transforming them in to volatile form and transferring into atmosphere (EEPA, 2000).

The treatment unit cell planted with Vetiver grass demonstrated higher performance in removing selected organic matter rather than cell planted with Nut sedge, because Vetiver grass has fine, massive, deep, spongy roots system which provides enormous surface area for bacterial growth and multiplication and the bacteria may break down the organic matter through aerobic or anaerobic process. The Vetiver's erect and stiff stem are used as bio filter, trapping fine and coarse sediments (Truong, 2008).

A study conducted by Christina (2006), showed maximum 58 %BOD removal and 73 % COD reduction in constructed wetland through different wetland plants for tannery waste water.

A similar study conducted by Ketema (2009), using tannery waste water in CW under 5 days of HRT showed maximum 84 % BOD removal and 68 % COD removal using sesbaniasesba and schnopplectuscorymbsus, respectively.

A similar study also conducted by Terfie and Asfaw (2015), using selected wetland plants for removing Cr and organic pollutants from tannery under 5 days of HRT showed that 77.9 % BOD removal and 80.9 % of COD removal using P.karka, from other selected plants (C. alternifolius, T. domingensis and B.aethiopium).

This study showed that, the reduction of organic pollutants in constructed wetland was higher compared to other study. The effluent BOD_5 and COD concentration from wetland treatment was achieved the permissible limit standards set by EEPA for tannery industry. The professional discharge limit set by EEPA (2003) to water bodies; is 200 mg/l, 500mg/l for $BOD_5$and COD respectively. This may due to; this study uses constructed wetland technology as post treatment system whereas the other researcher uses the constructed wetland as primary treatment plant.

4.2.2 Removal of heavy metals in constructed wetland

The concentration of heavy metals including Cadmium (Cd), copper (Cu), nickel (Ni), Zink (Zn) and lead (Pb) discharged from CW were very low. Both the influent and effluent value of heavy metals on constructed wetland was insignificant (<0.1mg/l), because the heavy metals except chromium are not consumed in manufacturing process in tanning industries.

4.2.2.1 Chromium removal in constructed wetland

The influent concentration of chromium to constructed wetland was 4.6mg/l and the average value of effluents after treatment was 0.107, 0.23 and 0.25 for Vetiver, Sedge and control treatment unit cell respectively. The removal efficiency of these experimental unit cells can be shown in figure below.

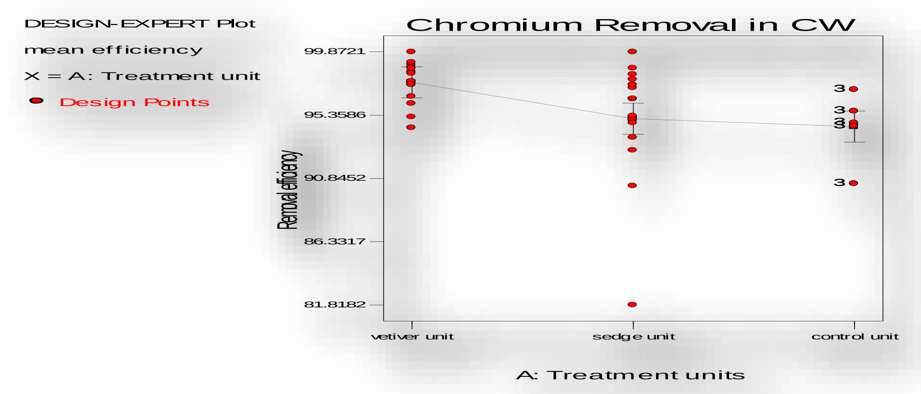

Figure 4.3 chromium removal in constructed wetland

From figure 4.3, the maximum chrome removal was recorded in experimental unit cell planted with Votive grass (97.7 %) and followed by sedge unit (95). The lower chrome reduction was recorded in control unit (94.5%). Statistical analysis ($P<0.05$) showed that, the Chromium removal in treatment unit cell planted with Vetiver grass was significantly higher than Nut sedge and control unit. Likewise organic matter, Votive grass demonstrated higher performance in removing Chromium rather than Nut sedge. This was due to morphological and physiological nature of Vetiver grass.

As shown in figure 4.3, there was no any statistically difference between sedge and control unit for chromium removal, because chromium was highly removed with aid of substrate and microorganism through adsorption, complexation and redox reaction process rather than vegetation.

The greatest removal efficiency was recorded on chromium metal compared to organic pollutants, because chromium is removed in constructed wetland through a complex process involving physical, chemical and biological mechanism with aid of substrate, vegetation and wetland microbes. Redox reaction, sorption, complexiation, and hydroxide precipitation, adsorption, cat ion exchange and plant uptake are the most dominant mechanism for remediating chromium metal in CW (Watson et al., 1989).

In adsorption and cat ion exchange mechanism, the positive charged particle is bind on the surface of negative sited of plants which has a cationic property through carboxyl functional group(-COOH) in humic acid of plant cellular tissue (Terfie and Asfaw,2015). The wetland plants filter, adsorb and precipitate chromium on its root zone through Rhizo filtration process. The chromium absorb, translocate to above portion of plant tissue through phytoextraction process (Etim, 2012).

The similar study conducted by Ketema (2009) and Terfie and Asfaw (2015), for tannery waste water treatment in constructed wetlands using wetland plants were fitted to this study result, but these two studies couldn't achieve the permissible limit standards of EEPA with respect to chromium discharged to the environment.

This study was used a constructed wetland technology as complementary treatment along with conventional treatment rather than as alternative treatment. It was used as post treatment method after primary treatment. As the result, the chrome effluent could meet the permissible limit standard, despising the other study.

4.2.3 The removal trends of organic and inorganic pollutants in constructed wetland

The performance of constructed wetland was also examined through trends of its removal efficiency. The average value of each consecutive batches should be taken for each consecutive trends of removal that shown in table below.

Table 4.2 the removal trend of selected pollutants in constructed wetland

Treatment unit	Trend	% BOD_5	% COD	% Cr
Vetiver unit	1	85.075766	77.23095	95.0231
Vetiver unit	2	86.884509	76.349826	95.977
Vetiver unit	3	88.1119649	80.850845	97.6673
Vetiver unit	4	91.2142876	88.037232	98.5081
Sedge unit	1	87.9792393	80.594098	92.0679
Sedge unit	2	86.5920975	80.750237	99.0982
Sedge unit	3	84.2930269	72.647985	96.6905
Sedge unit	4	84.2930269	66.150329	95.0357
Control unit	1	93.9214405	76.482812	96.4194
Control unit	2	87.984828	68.131303	94.6955
Control unit	3	82.3077514	72.20108	95.0057
Control unit	4	50.0324721	63.362299	93.9496

As shown table 4.2, the Vetiver unit showed increasing trends for removing organic and inorganic pollutants, the Sedge unit showed increasing and decreasing at the stage whereas the control unit demonstrated decreasing removal trends for each selected pollutants.

4.3 Growth performance and chromium accumulation capacity of Vetiver grass and Nut sedge

4.3.1 Morphological characteristics and growth performance of experimental plants

After feeding diluted waste water, both Vetiver grass and Nut sedge were grown rapidly and changed its color from pale green to dark green. The Vetiver grass tended to show continual growth throughout the experiment, whereas the Nut sedge growth intended to decrease after starting up experiment. The color of Nut sedge was changed to yellow pale and become chlorosis due to toxicity of heavy metal.

Figure 4.4 Vetiver grass and Nut sedge at mid time experiment.

Figure 4.5 Vetiver grass and Nut sedge at the end experiment

As shown figure 4.5, the Vetiver grass showed maximum stem height which rise to 160 cm within the seven months experimental periods and showed deep, massive, fibrous root system which used to tolerate adverse climatic condition and high level of heavy metal.

At the end of the experiment, the Vetiver grass was higher than Nut sedge in terms of stem height, root length, and dry weight throughout all replication unit cells. The average stem height, root length, dry weight of Vetiver grass was 128 cm, 71cm, 74cm respectively, whereas Nut sedge was 73.1cm, 26cm and 36cm per clump. The table below shows the morphological characteristics of experimental plants.

Table 4.3 average stems height, root length, dry weight of experimental plants

TN	Species	Stem height(cm)	Root length(cm)	Dry Wt(g)
1	Vetiver grass	128	71	74
2	Nut sedge	73.1	26	36

The analysis showed that, the stem height, root length, and dry weight of Vetiver grass was higher than sedge one. These due to the morphological nature Vetiver grass which tolerate unfavorable climatic condition and resist to toxic heavy metal.

The Nut sedge had higher number of tillers per clump than Vetiver grass throughout all replication unit cells. The average number of tillers of Nut sedge and Votive grass was shown in table below.

Table 4.4 average tillers per clumps and color of Vetiver grass and Nut sedge (Nut grass)

No	Species	No. tillers	Color
1	Vetiver grass	16	Green
2	Nut sedge	52	Yellow pale

4.3.2 Phytoaccumulation of chromium

To evaluate the phytoaccumulation capacity, both roots and shoots of Vetiver grass and Nut sedge were analyzed separately. The average chromium concentration (mg/kg) of Votive grass and Nut sedge is shown in table below.

Table 4.5 average chromium accumulation on experimental plant Tissues

No	Plant tissues	Vetiver grass (mg/kg)	Nut sedge(mg/kg)
1	Root	55.1	33.86
2	Shoot	13.9	4.1
3	Biomass	30.8	13.9

4.3.2.1 Comparison of Vetiver grass and Nut sedge for chromium accumulation

The statistical plant analysis showed that, there was a difference between Vetiver grass and Nut sedge through accumulation of total chromium in their plant tissues. The figure below shows the difference in accumulation of chromium retained with in experimental plants.

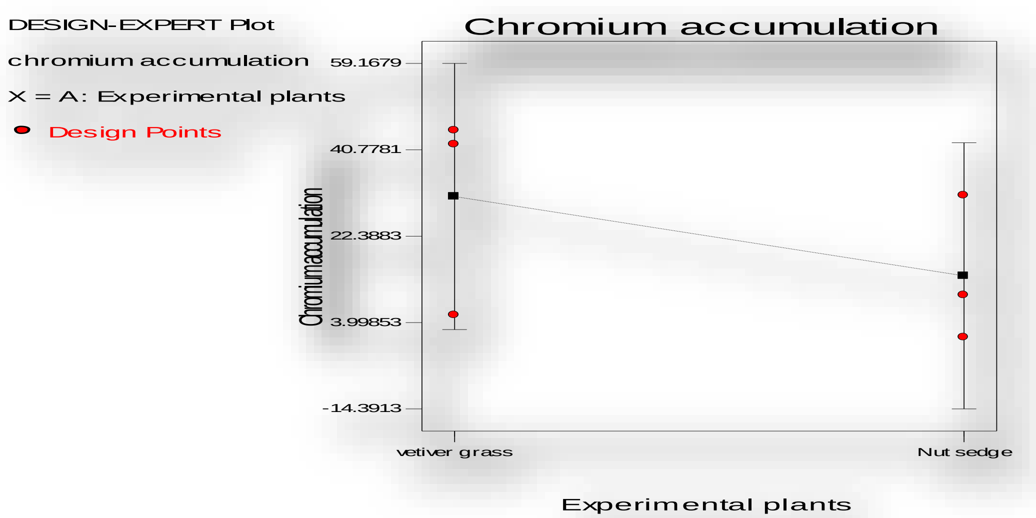

Figure 4.6 accumulation of chromium on experimental plants

Higher concentration of total chromium was recorded in Vetiver grass (30.8 mg/kg) compared to Nut sedge (13.9 mg/kg) in constructed wetland experiment. The result revealed that, the root and stem of Vetiver grass stored significantly higher amount of chromium metal than its tissue of Nut sedge. Physiologically, Vetiver grass resists the toxicity of heavy metals and different pH ranges such as acidity, salinity and basicity (Troung, 2008). As the result the Vetiver showed continual growth and development ignoring environmental stress. Morphologically, Vetiver grass has massive, long, fine and fibrous root which used to adsorb and accumulate chromium from sounding area. The stiff long stem of Vetiver grass may be used to stored chromium through phytoaccumulation and phytodegradation process (Etim, 2012). The Vetiver grass accumulates chromium, translocate and concentrate it to above ground parts and produce large biomass, whereas Nut sedge has shorter root system and lower resistant to chromium toxicity compared to Votive grass. At the end of the experiment, the growth of Nut sedge was retarded and its stem became chlorosis which is a sign of toxicity. This may due to the xylem, phylum and stomata of Nut sedge was inhibited through accumulation of chromium (Nagajyoti et al, 2010).

4.3.2.2 Comparison of root and stem of Vetiver grass and Nut sedge for chromium accumulation

In this study, the plant analysis revealed that, there was a great variation between stem and root capacity in accumulation of chromium for both experimental plants. The figure below shows the difference in capacity of root and stem for its chromium accumulation.

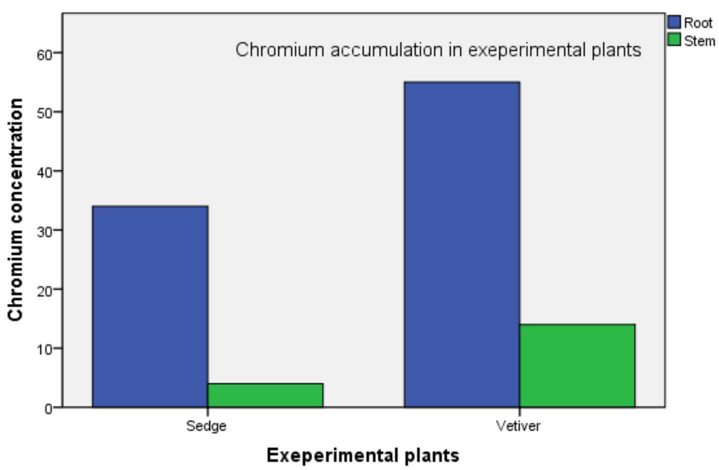

Figure 4.7 chromium accumulations in root and stem of experimental plants

The plant analysis showed that, the root of both Vetiver grass and Nut sedge retained higher amount of chromium concentration than its stem parts, because more chromium was accumulated in root surface through adsorption and cationic exchange process. In a cationic exchanged reaction, positively charged chromium ion in the solution bind to the negative charged sited on the surface of plant root through its carboxyl functional group (-COOH) in the humid acids of cellular tissue (Weisa and Weisb, 2003). According to Terfie and Asfaw (2015), Chromium can be adsorbed at extra cellular negative charged site (COO-) of root cell wall.

4.3.2.3 Bioaccumulation and translocation capacity of experimental plants

A. Bioaccumulation factor

Bio accumulation factors (BAF) is an index which used to determine the capacity of plants for absorbing and accumulating heavy metals with respect to sounding medium. The figure below shows the bioaccumulation factor ofexperimental plants in constructed wetlands.

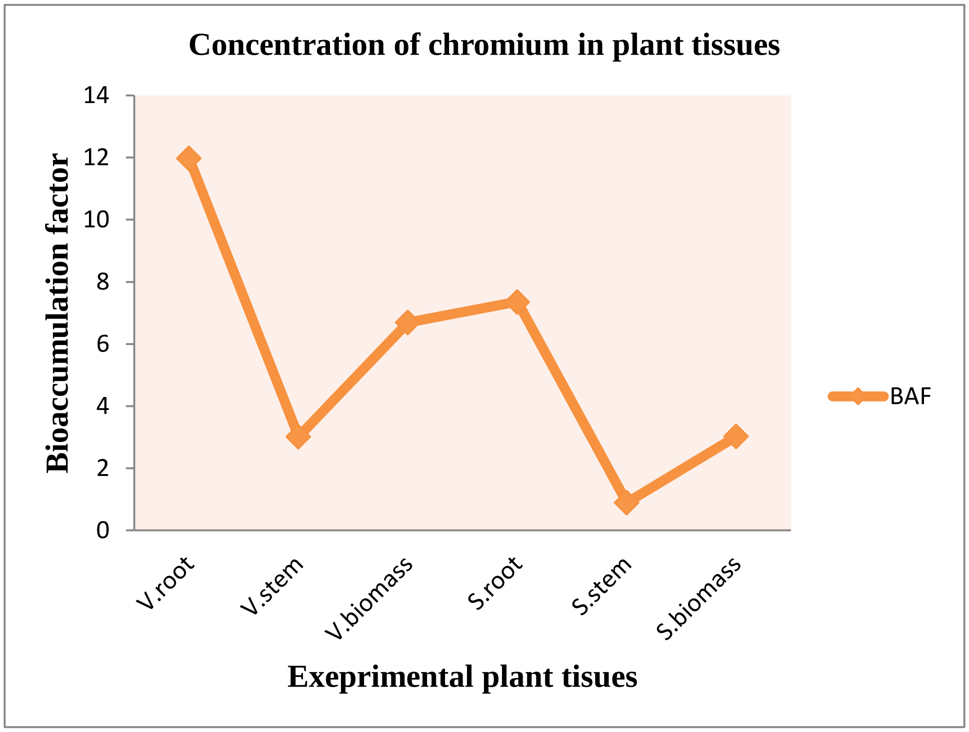

Figure 4.8 bio accumulation capacity of experimental plants

From figure 4.8, it can be seen that, the highest bioaccumulation factor was recorded in root of Vetiver grass (12 times) and the least BAF was recorded in stem of Nut sedge (0.9 times) with respect to the sounding water. The bioaccumulation factor (BAF) of Vetiver root and stem was higher than Nut sedge one. The bioaccumulation factor of Vetiver root and stem were 12 times and 3.01 times respectively, whereas the root and stem of Nut sedge were 7 times and 0.9 times, respectively. Both biomasses BAF value of Votive grass (6.7 times) and Nut sedge (3 times) was greater than two. The higher the BAF value (BAF >2) implies the plant suitable for phytoextruction and greater in phytoaccumulation capacity (Mellem et al., 2009). According to Ghejua (2009) and Tarfie and Asfaw (2015), the plants BAF >1 will remove metal in waste water with each plant. So, the experimental plants have potential to remove chromium in tannery waste effluent.

B. Translocation factor

The capacity of plants for absorbing and translocating heavy metals from root to above ground of plant is estimated using translocation factor. The translocation capacity of experimental plants is shown below in the figure.

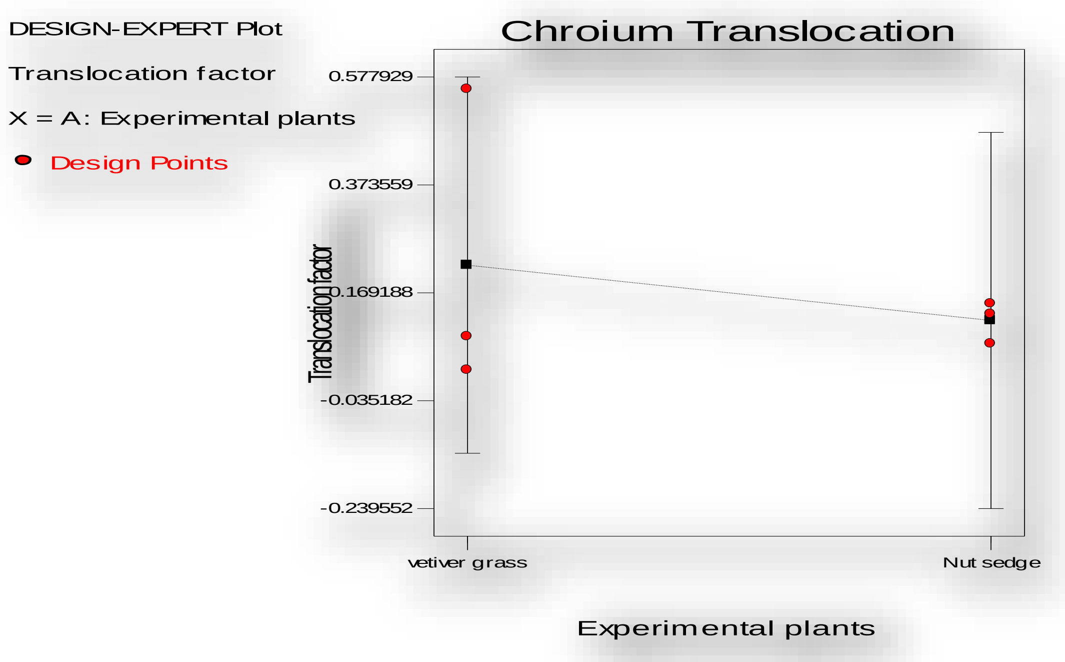

Figure 4.9 Translocation of chromium from root to shoot.

As shown in figure, the translocation capacity of Vetiver grass for chromium (0.25) was higher than Nut sedge (0.12).The translocation factor value of both experimental plants was less than one, because much chromium was accumulated in root tissue rather than stem. The translocation capacity of plants depend on different factors including rate of xylem loading/translocation to roots, proportion of metals fixed with root, rate of metal uptake by roots and cellular tolerance to toxic metals. The translocation and accumulation capacity of plant is related to its ecotype (Etin, 2012).

Chapter five

Design proposed wetland for scale up the pilot scale

The Batu tannery factory produces leather products from skin and animal hid and generate proportional amount of waste water. The factory generates and discharge 150m^{3}volume of waste water per day. Based on this design discharged flow rate, the wetland treatment will be designed as post treatment plant. This proposed constructed wetland should be refreshed with tap water and also trim the wetland plants during the maturation stage.

5.1 Land requirement for proposed wetland

The design flow rate and HRT are the given parameters that are already designed

Q=150 m^3/day (design flow rate) and HRT=7 days (from experiment)

Assume

$Aspect\ ratio = 5:1 = L:W$

$Porosity = 35\%$

$Media\ depth = 0.5m$

Wetland depth =media depth+ Safety factor (25%)= $0.63m$

$Water\ depth = 0.45m$

$Slop = 1\%$

Scale factor =1: 64

The total amount of area required for treatment can be calculated as by using Darcy law:

$HRT = \frac{V}{Q}$ (Darcy law)

$HRT = \frac{LWdn}{Q}$ where, H=hydraulic retention time

$L =\ length\ (m)$

$W = \ width\ (m)$

$d = depth\ (m)$

$n = porosity\ (35\ \%)$

$Q = \ design\ flow\ rate\ (m3/\ day)$

$$HRT \quad = \frac{LWdn}{Q} \ = \frac{5W \times}{Q} W \times dn \quad since, L = 5W$$

$$HRT = 5W \times \frac{dn}{Q} W$$

$$W = \sqrt{(}HRT \times \frac{Q}{5dn}) \ = \sqrt{(}7\ day \times \frac{150\,\frac{m3}{day}}{5\ \times 0.35 \times 0.45m}) \ = \sqrt{(}1333.3m2) \ = 36.5\ m$$

$Hence, L \ = 5W \ = 182.5m$

$Therefore, Area\ required\ (A) = L \times W \ = 36.5.m \times 182.5m \ = 6661\text{m}^2$

5.2 Sizing

The materials that used to construct design wetland and their size are discussed below.

a) Gravel medium : the gravel needed for constructing design wetland can be calculated as:

$Vg = L \times W \times d = \ 6661\ m2 \times 0.5m = 3330\ \text{m}^3$. Hence, total 3330 m^3 gravel is required.

b) Blokes: the size of one block is 0.20m

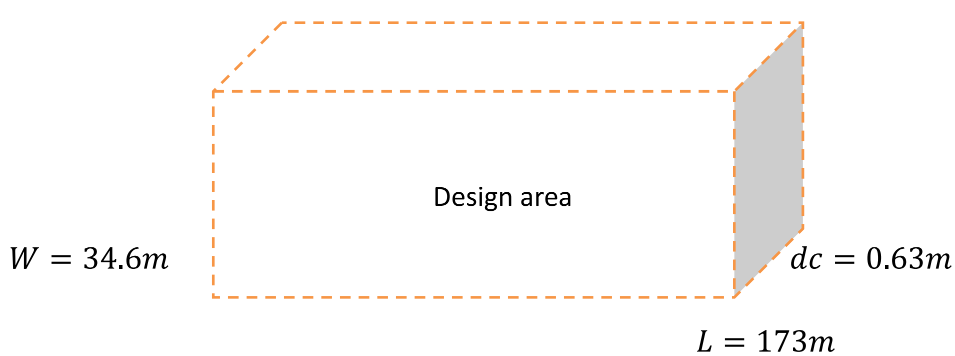

Figure 5.1 design wetland

Total blocks needed in row(X) direction = $\frac{length\ of\ CW}{length\ of\ unit\ block} = \frac{2L}{0.2m}$

$$= \frac{2 \times 182.5m}{0.2m} = 1825\ blocks$$

Total blocks needed in column(Y) direction= $\frac{width\ of\ CW}{length\ of\ unit\ block} = \frac{2W}{0.2m}$

$$= \frac{2 \times 36.5m}{0.2m} = 365\ blocks$$

Total blocks needed in up (Z) direction = $\frac{depth\ of\ CW}{depth\ of\ block} = \frac{0.625m}{0.2m}$

$$= 3\ blocks$$

Total blokes needed for design $wetland\ = (1825 + 365) \times 3blocks$

$$= 6570\ blocks$$

c) Liner: the liner is sealed on the design wetland. So, it is equal the sum of design area and the area of wetland side.

$Total\ liner\ required\ = 6661\text{m}^2 + 0.625m \times 2 \times 36.5m + 0.625 \times 2 \times 182.5m$

$=6935\ \text{m}^2$

d) Green house plastic: the size of green house plastic is the same as the area of design wetland

$$plastic\ size = A \times safety\ factor$$

$= 6661\ m2 + 0.25 \times 6661\text{m}^2$

$= 8326\text{m}^2$

The size of materials that used to design wetland summarized in table below.

Table 5.1 size of construction materials

No	Materials	Units	Size(quantity)
1	Gravel	m^3	3330
2	Pvc pipe	M	100
3	Plastic shad	m^2	8326
4	Gate valve	Pic	2
5	Elbow	Pic	2
6	Liner	m^2	6935
7	Cement	Kg	5000
8	Sand	m^3	450
9	Blocks	Pic	6570

5.3 Material purchased cost

The cost of materials can be described in below table.

Table 5.2 purchased cost of materials

No	Material	Size(quantity)	Unit cost	Total cost
1	Gravel	3330	$14.5	$48,285
2	Pvc pipe	100	$2.5	$250
3	Plastic shade	8326	$1.3	$10,823
4	Gate valve	2	$5.4	$10.8
6	Elbow	2	$5.4	$10.8
7	Fittings	74	2.5	$185
8	Cement	5000	$4.5	$22500
9	Sand	450	$22.5	$10125
10	Blocks	6570	$0.76	$4993
		Sum total		**$97,182.6**

Assume the total purchased equipment cost is 15-40% of fixed capital investment.

Purchased equipment cost $(PIC) \; = 15 - 40\,\%\, FCI \; = 0.27 \times FCI$

$FCI = \; PIC/0.27 = \$97{,}182.6/0.27 \; = \$359{,}935.5$

Thus, total amount of fixed capital investment for completing design wetland is $\$359{,}935.5$

5.4 Cost benefit analysis

Not only the fixed capital investment, but also the operational and maintenance cost of wetland plant is very low. Despising land acquisition, the wetland technology has many benefits including hydrological benefits, ecological benefits, educational and recreational benefits.

Generally, constructed wetland technology is so cost effective and feasible compared to conventional treatment methods.

Chapter six

Conclusion and recommendation

6.1 Conclusion

The aim of this study was to examine the Phytoremediation capacity of Nut sedge and Vetiver grass for removing selected organic and inorganic pollutants in Horizontal sub surface flow constructed wetland. The results obtained in this study lead to the following conclusions.

The waste water characterization showed that the concentration of Cr and other selected pollutants which discharged from selected industry are much beyond the permissible limit standards set by EEPA (2003). The total Chromium is the dominant pollutant in the selected industry rather than other heavy metals which are insignificant value.

The waste water analysis showed that, the BOD, COD, TSS and Cr of effluents were reduced to 88%, 80.8%, 80.4 % and 97.7 % respectively in constructed wetland planted with Vetiver grass, whereas 78.5% BOD, 73.1% COD, 69.04% TSS and 94.5% of Cr were reduced in control unit. The statistical analysis ($P< 0.05$) showed that, there was a significance difference between the phytoremediating experimental units and control unit for removing these selected pollutants, due to vegetation which provided remediating pollutants through different mechanisms including Rhizodegradation, Rhizofiltration, phytodegradation and stabilization (Etim, 2012).

Due to the morphological and physiological nature of Vetiver grass the treatment unit cells planted with Vetiver grass showed that the highest performance in removing Cr and selected organic matter rather than cell planted with Nut sedge.

Except TSS, the concentration of all wetland effluents from this study were below the permissible limit standards set by EEPA (2003).

The chromium metal was largely accumulated in root tissues of both experimental plants. The bioaccumulation capacity (6.7 times) and translocation capacity (0.25) of Vetiver grass was higher compared to the Nut sedge which had 3times of BAF and 0.12 times of TF.

It can conclude that, constructed wetland treatment using Vetiver grass is going to be candidate as alternative solution through post treatment method for small scale tannery industry.

6.2 Recommendation

1. It is recommended that the constructed wetland treatment should be scale up and used as Secondary treatment rather than as a primary treatment method to comply environmental legislation.

2. Further study should be taken on gravel medium and wetland plants to reuse again in the process during its maturation stages.

3. It is recommended that the industries should pay attention on position of constructed wetland when build it, since the treatment is performed with gravitational energy.

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Annexes

Annex-1:- waste water result before and after CW compared with EEPA limit standards.

Parameters	**Influent (mg/l)**	**Treatment unit**	**Effluent results(mg/l)**	**Permissible limit(mg/l) (EEPA 2003B)**	**Removal efficiency (%)**
BOD	503	Vetiver unit	60.36	200	88
		Sedge unit	72.4		85.6
		Control unit	108.1		78.5
COD	1046.4	Vetiver unit	201.4	500	80.8
		Sedge unit	267.4		74.5
		Control unit	281		73.1
TSS	670.4	Vetiver unit	130	50	80.5
		Sedge unit	140.8		79
		Control unit	207		69.04
Cr	4.6	Vetiver unit	0.107	2	97.7
		Sedge unit	0.23		95
		Control unit	0.25		94.5
HM(Pb,Cu,Ni, Cd& Zn)	<0.1	Vetiver unit	<0.1	_	-
		Sedge unit	<0.1		-
		Control unit	<0.1		-

Annex-2:ANOVA for COD on Vegetative units(Vetiver and Sedge) and control unit

Response: Efficiency

ANOVA for Selected Factorial Model

Mean	std.Dev.	C.V
75.5	9.5	12.6

Treatment unit	Estimated Mean	Standard Error

1-Vetiver unit	80.77500975	2.455385109
2-Sedge unit	74.46555562	2.455385109
3-Control unit	71.3908897	2.455385109

Treatment unit	Mean Difference	DF	Standard Error	t for H0 Coeff=0	Prob> |t|
Vetivervs Sedge	6.309454134	1	3.472438922	1.817009	0.0764
Vetivervs Control	9.384120059	1	3.472438922	2.702458	0.0099
Sedge vs Control	3.074665925	1	3.472438922	0.885449	0.3810

Values of "Prob> |t|" less than 0.0500 indicate the difference in the two treatment means is significant.
Values of "Prob> |t|" greater than 0.1000 indicate the difference in the two treatment means is not significant.

Annex-3:-ANOVA for BOD on Treatment unit

Response: Efficiency

ANOVA for Selected Factorial Model

Mean	Std.Dev.	C.V
86.7	0.97	1.12

Treatment efficiency Means

	Estimated Mean	Standard Error
1-Vetiver unit	87.8929	0.2517
2-Sedge unit	85.6065	0.2517

Treatment	Mean Difference	DF	Standard Error	t for H0 Coeff=0	Prob> |t|

Vetivervs Sedge	2.28643	1	0.35596	6.42335	< 0.0001

Values of "Prob> |t|" less than 0.0500 indicate the difference in the two treatment means is significant.

Values of "Prob> |t|" greater than 0.1000 indicate the difference in the two treatment means Isnot significant

Annex-4:-ANOVA for Cr on Vegetative unit cells(Vetiver and Sedge) and Control unit cell

Response: Removal efficiency mean

ANOVA for Selected Factorial Model

Mean	std.Dev.	C.V
95.8	3.01	3.144

Analysis of variance table

Treatment efficiency Means

	Estimated Mean	Standard Error
1-Vetiver unit	97.6971	0.77755
2-Sedge unit	95.1087	0.77755
3-Control Unit	94.5412	0.77755

Treatment Unit	Mean Difference	DF	Standard Error	t for H0 Coeff=0	Prob> |t|
Vetivervs Sedge	2.58838	1	1.09962	2.35388	0.0233
Vetivervs Control	3.15592	1	1.09962	2.87	0.0064
Sedge vs Control	0.56754	1	1.09962	0.51612	0.6085

Values of "Prob> |t|" less than 0.0500 indicate the difference in the two treatment means is significant.

Values of "Prob> |t|" greater than 0.1000 indicate the difference in the two treatment means is not significant.

Annex-5:-TSS ANOVA on Vegetative unit cells (Vetiver and Sedge) and Control unit cell

Response: Removal efficiency mean

ANOVA for Selected Factorial Model

Mean	std.Dev.	C.V
76.1	26.05	34.2

Treatment efficiency Means

	Estimated Mean	Standard Error
1-vetiver unit	80.48157	6.725615
2-sedge unit	78.99189	6.725615
3-control unit	69.04091	6.725615

Treatment unit	Mean Difference	DF	Standard Error	t for H0 Coeff=0	Prob> |t|
vetivervssedge	1.489686	1	9.511456	0.15662	0.8763
vetivervscontrol	11.44066	1	9.511456	1.20283	0.2358
sedgevscontrol	9.950977	1	9.511456	1.04621	0.3014

Values of "Prob> |t|" less than 0.0500 indicate the difference in the two treatment means is significant.

Values of "Prob> |t|" greater than 0.1000 indicate the difference in the two treatment means is not significant.

Annex-6:-Significance level of ANOVA for selected Parameters**(using SPSS Program version 20)**

Parameters	Relation	Sum of Squares	df	Mean Square	F	Sig.
BOD	Between Groups	686.178	2	343.089	1.310	.281
	Within Groups	10997.733	42	261.851		
COD	Between Groups	1116.978	2	558.489	5.684	.007
	Within Groups	4126.667	42	98.254		
TSS	Between Groups	1165.378	2	582.689	.860	.430
	Within Groups	28448.533	42	677.346		
CR	Between Groups	82.978	2	41.489	4.325	.020
	Within Groups	402.933	42	9.594		

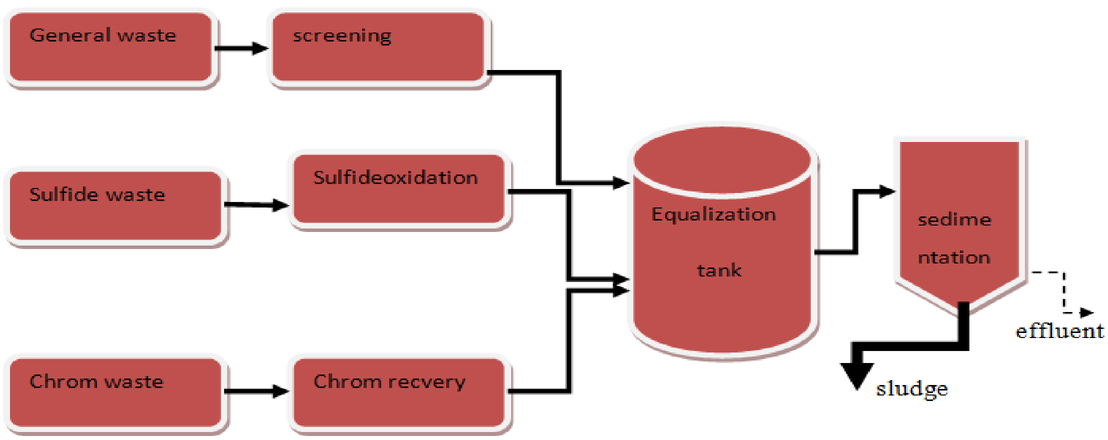
Annex-7:- Heavy metal accumulation in pre experimental plants (mg/kg)

Plants	Cr	Cu	Zn	Pb	Cd	Ni
Votive	0.1271	0.3443	0.169	0.1517	0.031	0.0782
Sedge	0.0543	0.2228	0.8593	0.105	0.039	0.1054

Annex -8:-Average Heavy metal accumulation in post experimental plant species

plant species	Cr(mg/kg)	Cu(mg/kg)	Zn(mg/100kg))	Pb(mg/kg)	Cd(mg/kg)	Ni(mg/100kg))
Vetiver grass	30.8333	0.40333	1.98667	0	0.05667	0.75333
Nut Sedge	13.9433	0.16	2.57333	0.14333	0.14	0.21

Anex-9:-Waste water treatment line for Batu tannery factory



Annex-10:-Production process op tannery factory